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# ENZYMES OF CARBOHYDRATE METABOLISM IN DEVELOPING *HORDEUM DISTICHUM* GRAIN

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**Key Word Index**—*Hordeum distichum*, Gramineae, barley, endosperm, amyloplast, carbohydrate metabolism, GIP synthesis

**Abstract**—Variations in activity of several enzymes associated with carbohydrate metabolism were recorded during the development of barley endosperm The enzymes investigated were sucrose-UDP (ADP) glucosyl transferase, invertase, UDPG (ADPG) pyrophosphorylase, hexokinase, glucose-6-phosphate ketoisomerase, phosphoglucomutase, and nucleosidediphosphokinase

## INTRODUCTION

INITIATION of short-chain glucan primers may be carried out by phosphorylase (E.C 2 4 1.1), with glucose-1-phosphate (GIP) serving as the glucosyl donor [1,2] The nucleotide sugars, UDPG (UDP-glucose) and ADPG (ADP-glucose) which, with these primers, are the substrates for starch synthesis[3,4] may be synthesized from sucrose by sucrose-UDP (ADP) glucosyl transferase (E C 2 4 1 13),[5] or from GIP by ADPG (UDPG) pyrophosphorylase (E C 2 7 7 9) [6] GIP, itself a product of sucrose metabolism, is thus a key intermediate in the conversion of sucrose to starch, and variations in its concentration may provide a regulatory mechanism in starch biosynthesis Enzyme systems concerned in the biosynthesis and metabolism of GIP and the nucleotide sugars were therefore investigated in barley endosperm, in order to determine changes in activity during maturation

## RESULTS

### *Major Biochemical Constituents*

The relative contributions of starch, reducing sugars, and protein to the dry weight of the grain, and of water to the fresh weight, are shown in Fig 1 The proportion of reducing sugars fell as starch accumulated in the endosperm Protein levels increased during the first 20 days after anthesis, and then remained fairly constant during maturation Water content was high in the young grain, reaching a maximum around 14 days after anthesis, then falling steadily as the grain ripened The levels of biochemical constituents described here were approximately the same as those in the grain used for enzyme assays (see below)

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*Sucrose-UDP (ADP) Glucosyltransferase*

Activity with UDP, which was considerably greater than with ADP in soluble endosperm extracts, could be detected at 5–7 days, and increased rapidly until 25 days (Fig 2) Activity with ADP was not apparent until 8 days, and rose gradually to a maximum around 34 days No activity with either UDP or ADP was detected in amyloplast fractions

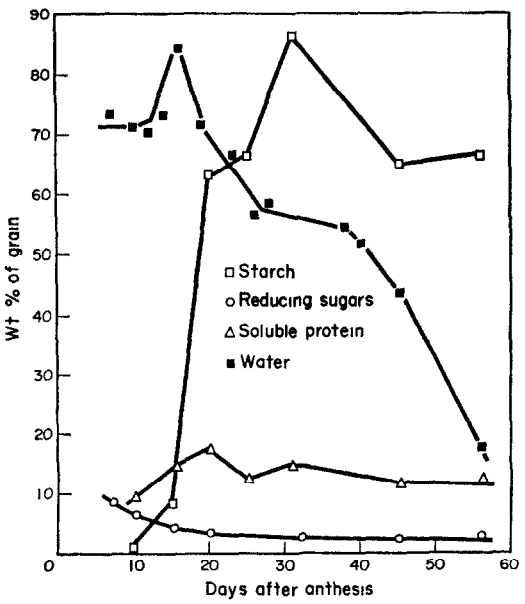


Fig 1. Starch, soluble reducing sugars and total soluble protein as a % of dry wt, and water as a % of fr wt of whole endosperm during maturation

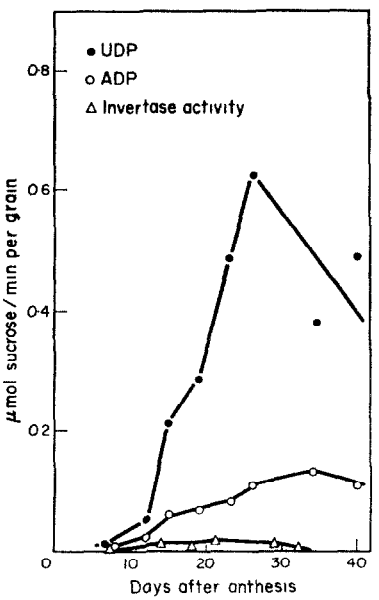


Fig 2 Sucrose—UDP (ADP) glucosyl transferase activity with UDP and ADP, and invertase activity in soluble endosperm extracts of developing endosperm

*Invertase* (E C 3 2 1 26)

This was measured both at pH 4 8 and 7 2, and activity was low in each case The variation in activity at pH 7 2 throughout maturation in the soluble fraction of endosperm is shown in Fig 2 Activity was detected around 10 days after anthesis and remained fairly constant during maturation, declining after 30–34 days By 21 days after anthesis a significant proportion of the total grain activity was located in the soluble fraction of the embryo

*UDPG (ADPG) Pyrophosphorylase*

The UDPG enzyme could be detected in the soluble endosperm fraction by 7 days after anthesis Activity increased rapidly at first, levelling off around 16 days, then rising again to a peak at 24 days (Fig 3) The ADPG enzyme appeared somewhat later—around 12 days, and a maximum activity, slightly lower than that of the UDPG enzyme, was reached by 21 days

*Inorganic Pyrophosphatase* (E C 3 6 1.1)

Figure 4 shows that activity in the early stages of maturation closely resembled the pattern of ADPG pyrophosphorylase Maximum activity, however, was attained around the same

period as UDPG pyrophosphorylase Activity was chiefly located in the soluble fraction, but some could be detected in the amyloplasts from 12 days after anthesis

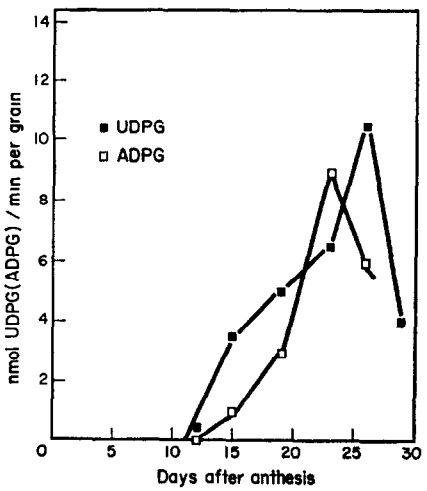


FIG 3 UDPG AND ADPG PYROPHOSPHORYLASE ACTIVITY IN SOLUBLE ENDOSPERM EXTRACTS.

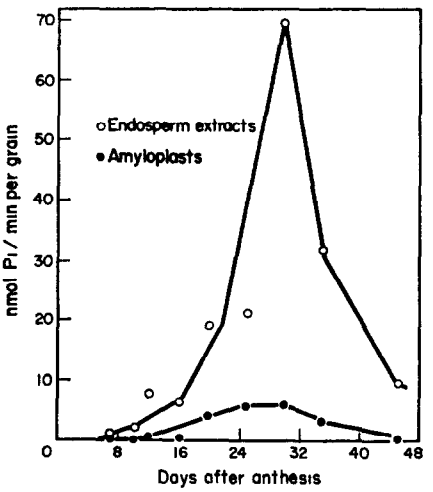


FIG 4 INORGANIC PYROPHOSPHATASE ACTIVITY IN SOLUBLE ENDOSPERM EXTRACTS AND AMYLOPLASTS

*Hexokinase* (E C 2 7 1 1)

This enzyme was present in both soluble and amyloplast fractions from 7 days after anthesis and increased steadily throughout the initial stages of maturation, levelling off around 21 days Similar results were obtained with glucose and fructose as substrates The results expressed in Fig. 5 were obtained using glucose. Amyloplast activity was approximately half that of the soluble fraction

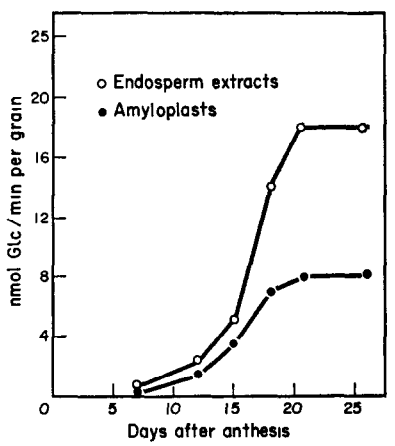


FIG. 5. HEXOKINASE ACTIVITY IN SOLUBLE ENDOSPERM EXTRACTS AND AMYLOPLASTS.

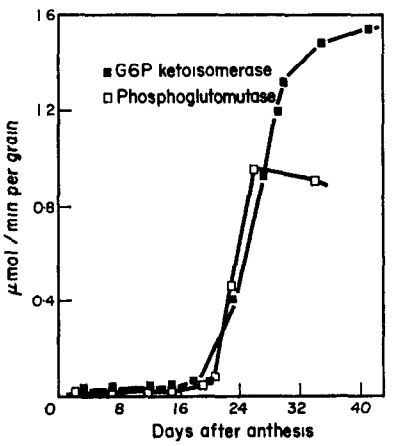


FIG. 6 G6P KETOISOMERASE ACTIVITY IN μmol F6P/min/10 grains AND PHOSPHOGLUCOMUTASE IN μmol $P_i$/min/10 grains (VALUES × 2) IN SOLUBLE ENDOSPERM EXTRACTS

*Phosphoglucomutase* (E C 2 7 5 1) *and Glucose-6-phosphate ketoisomerase* (E C 5 3 1 9)

Both enzymes were present in the soluble endosperm extract very early in development, and exhibited similar patterns of activity (Fig 6) There was no appreciable change until 18 days, when activity increased sharply, between 20 and 22 days Glucose-6-phosphate (G6P) ketoisomerase activity began to level off around 25 days, when phosphoglucomutase was already declining

*Nucleoside diphosphokinase* (E C 2 7 4 6)

Sucrose-UDP glucosyl transferase and ADPG pyrophosphorylase exhibit their greatest increase in activity around 15–20 days after anthesis Nucleoside diphosphokinase was therefore assayed in soluble endosperm extracts of this age to determine whether interconversion of the nucleotides formed in these reactions was possible The presence of the enzyme was confirmed, and it was found to be more active with UTP (7 5 nmol/min/grain) than with GTP (4 5 nmol/min/grain)

## DISCUSSION

Patterns of changes in reducing sugars closely resembled those recorded in wheat[7] and peas,[8] and are consistent with a mechanism by which starch is synthesised from a precursor pool of soluble reducing sugars That is, the pool is initially depleted during the onset of rapid starch synthesis but is subsequently maintained at a constant (but lower) level, presumably by the continuing supply of sucrose to the grain [7] The increase in protein levels which immediately precedes the period of rapid starch accumulation may reflect synthesis of enzyme protein, since an increase in the activity of most enzymes investigated was observed during this stage of development However, maintenance of protein concentrations during later maturation may be attributed to the accumulation of protein bodies in the starchy endosperm [9]

The initial stages of starch synthesis, namely the formation of GIP and nucleotide sugars from translocated sucrose, appear to be localized in the soluble fraction of the endosperm, rather than in the amyloplasts The relatively high activity of sucrose-UDP glucosyl transferase in young endosperm suggests that most of the sucrose entering the endosperm is converted to nucleotide sugars, predominantly UDPG This contrasts with the situation in maize, where sucrose-UDP glucosyl transferase cannot be detected until 12 days after anthesis [10] In this case sucrose is thought to be metabolized to glucose and fructose via invertase, at least in the early stages of development [11]

Fructose is also a product of the sucrose-UDP glucosyl transferase reaction, and may be converted to GIP via hexokinase, G6P ketoisomerase and phosphoglucomutase activity,[12,13] since these enzymes are all present in barley endosperm soon after anthesis In the young endosperm GIP synthesized in this way may be immediately polymerized by phosphorylase activity, forming short-chain α-1,4-glucans which could serve as primers for transglucosylase activity [1,2,14]

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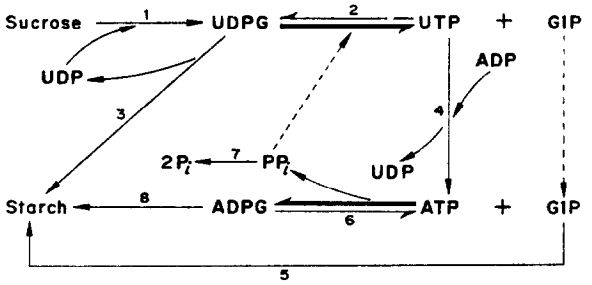
Some of the UDPG formed by sucrose-UDP glucosyl transferase activity may be converted to starch by UDPG-linked starch synthetase. However, during the period from 10 to 15 days after anthesis, increasing UDPG pyrophosphatase activity, in conjunction with low levels of inorganic pyrophosphatase would favour pyrophosphorylysis of UDPG, forming UTP and GIP Thus sucrose could be converted to GIP as proposed by Turner *et al*,[6] and De Fekete and Cardini [12] The subsequent increase in ADPG-pyrophosphorylase and inorganic pyrophosphatase would facilitate transfer of glucose from GIP to ADPG and thence to starch via ADPG-linked starch synthetase Detection of nucleoside diphosphokinase in endosperm extracts indicates that UTP formed from UDPG (via UDPG pyrophosphorylase) may be utilized to reform the ATP-required for ADPG synthesis




SCHEME 1 PROPOSED SCHEME OF SYNTHESIS OF STARCH FROM SUCROSE IN *Hordeum distichum* ENDOSPERM

1 Sucrose-UDP glucosyl transferase
2 UDPG pyrophosphorylase
3 UDPG-starch synthetase
4 Nucleoside diphosphokinase
5 Starch phosphorylase
6 ADPG pyrophosphorylase
7 Inorganic pyrophosphatase
8 ADPG-starch synthetase

In the reaction scheme outlined in Scheme 1, it is suggested that starch synthesis in very young endosperm is initiated by phosphorylase activity (using GIP as glucosyl donor), together with UDPG starch synthetase Coupling of the UDPG and ADPG pyrophosphorylase reactions would allow rapid conversion of sucrose to starch, via ADPG starch synthetase This would agree with previous results in which UDPG starch synthetase appeared earlier in development than the ADPG enzyme [15]

## EXPERIMENTAL

*Plant material* The 2-row barley *Hordeum distichum* (L) Lam C V Maris Baldric was used throughout. Conditions of growth and methods used to determine the date of anthesis were as described by Merritt and Walker [16]

*Preparation of soluble endosperm extracts and amyloplast fractions* The husk and testa-pericarp were removed by hand and the remaining endosperm (including the aleurone layer) together with the embryo constituted the 'whole grain' subsequently referred to This must be distinguished from the 'whole grain' of several other workers (for example, Jennings and Morton[7]) which includes the testa pericarp To prepare soluble endosperm extracts, the 'whole grain' was suspended in the appropriate buffer at 4° and homogenized by hand in an all-glass homogenizer The homogenate was filtered through muslin to remove cell debris and centrifuged at 4° for 10 min at 10 000 *g* The supernatant solution formed the soluble endosperm extract The pelleted material was washed once with buffer, re-centrifuged and re-suspended in buffer This fraction was composed mainly of amyloplasts and is referred to as the amyloplast fraction It was routinely examined by light microscopy and the age of the grain correlated with amyloplast diameter The number of grains used for extraction was varied from 60 (at 2–3 days) to 10 (18 days onwards) per 2 ml buffer The buffer required in the subsequent assay system was used as the extraction medium in each case

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*Chemical analyses* Reducing sugars,[17,18] carbohydrate,[19] and soluble protein[20] were measured by standard methods

*Enzyme assays* Sucrose-UDP glucosyl transferase[21] and invertase,[10] were assayed by increase in reducing sugars ADPG (UDPG) pyrophosphorylase,[8] hexokinase,[10] and nucleoside diphosphokinase[22] were estimated spectrophotometrically by coupling a product of the reaction to the G6P dehydrogenase reaction [8] Inorganic pyrophosphatase,[23] phosphoglucomutase[10] and G6P ketoisomerase,[10] were estimated by standard colorimetric methods

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